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Paleoenvironmental conditions in the Spanish Miocene–Pliocene boundary: isotopic analyses of *Hipparion* dental enamel

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Abstract Expansion of C_4 grasses during Late Miocene and Early Pliocene constitutes one of the most remarkable biotic events of the Cenozoic era. The Teruel–Alfambra region (northeastern Spain) contains one of the most complete Miocene–Pliocene sequences of mammalian fossil sites in the world. In this study, stable isotope ($\delta^{13}C$ and $\delta^{18}O$) analyses have been performed on the tooth enamel from the equid *Hipparion* from 19 localities spanning a time interval from approximately 10.9 to 2.7 Ma. This time range starts with the first appearance of this genus in Spain and ends at its extinction. An increase in $\delta^{13}C$ at about 4.2 Ma has been observed, indicative of a shift toward a more open habitat. This shift may be related to a large scale vegetation change which occurred across the Miocene–Pliocene boundary when C_4 grasses expanded. This expansion might in turn be linked to global tectonic events such as the uplift of the Himalaya and/or the closure of the Panama Isthmus. However, other more regional factors may have ultimately enhanced the trend toward more open habitats in the Western Mediterranean Basin. The Messinian Salinity Crisis was a major environmental event that may have been responsible for the isotopic changes seen in the equid *Hipparion* from the Iberian Peninsula along with an increase in the aridity detected ~4.6 Ma ago in the Sahara. Even though the exact factor triggering the isotopic change observed in the *Hipparion* enamel remains mostly unknown, this study demonstrates that the global environmental changes detected across the Miocene–Pliocene boundary are also recorded in the realm of the Iberian Peninsula.

Keywords *Hipparion* · Carbon and oxygen stable isotopes · Miocene–Pliocene boundary · Teruel–Alfambra region

Introduction

The carbon isotope composition of enamel from mammal herbivores has been used to distinguish between different types of paleodiets as it faithfully records the isotopic composition of consumed plants (DeNiro and Epstein 1978; Lee-Thorp and van der Merwe 1987; Quade et al. 1992), which depends on the photosynthetic pathways they use. According to this, plants can be grouped into three different photosynthetic pathways: C_3, C_4, and crassulacean acid metabolism (CAM). C_3 plants (85% of all terrestrial plants) use the Calvin photosynthetic pathway and include trees, shrubs, forbs, and cool-season grasses. C_4 plants (5–10% of all terrestrial plants) use the Hatch–Slack photosynthetic pathway and are mainly comprised of tropical, warm-season sedges, and grasses. Finally, the CAM photosynthetic pathway is used by a group of plants adapted to dry

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conditions. C_3 plants discriminate more against the heavy isotope (^{13}C) during photosynthetic fixation of CO_2 compared to C_4 plants. This results in very different carbon isotopic compositions between these two kinds of photosynthetic pathways. $\delta^{13}C$ values of C_3 plants range from −34‰ to −23‰ (VPDB), with an average of −27‰, whereas C_4 plants have $\delta^{13}C$ values ranging from −17‰ to −9‰ (VPDB), with an average of −13‰ (Bender 1971; Farquhar et al. 1989). CAM plants show intermediate carbon isotopic values. Nevertheless, this group of plants is not very frequently taken into account in paleodietary studies as they have rarely constituted an important food source for herbivore mammals.

Cerling and Harris (1999) proposed an enrichment in ^{13}C between tooth enamel and diet of ~14‰ in such a way that tooth enamel from herbivores feeding on C_3 vegetation have $\delta^{13}C$ values between −16‰ and −9‰, whereas those eating C_4 grasses display $\delta^{13}C$ values between −1‰ and +3‰.

Oxygen isotopes from enamel apatite have been used to obtain information regarding paleotemperatures and the evolution of past climates (Ayliffe et al. 1992; Sánchez Chillón et al. 1994; Lécuyer et al. 2003). The $\delta^{18}O$ value in both enamel phosphate and carbonate is related to that of body water, which in turn record water uptake (inspired O_2, drinking water, and plant water) and loss (excretion, expired CO_2, and water vapor) during tooth development (Bryant and Froelich 1995). By analyzing $\delta^{18}O$ values from herbivore tooth enamel, it is possible to differentiate between obligate drinkers (animals that obtain most of their water from drinking, frequently grazers) and non-obligate drinkers (those obtaining water mainly from plants, frequently browsers; Kohn 1996; Levin et al. 2006). However, when analyzing only one genus (as occurs in this study), enamel $\delta^{18}O$ can be used to track changes in the isotopic composition of meteoric water, which in turn is positively correlated to mean annual temperature (Dansgaard 1964; Rozanski et al. 1993). In this way, an increase in temperature and aridity is associated with an enrichment in the heavy isotope (^{18}O), whereas a decrease in temperature and greater humidity results in a depletion of the heavy isotope (Koch et al. 1989; Ayliffe et al. 1992).

A significant shift in global terrestrial environments occurred during late Miocene and early Pliocene epochs (between ~7 to ~5 Ma) characterized by the expansion of C_4 grasses. The predominance of the C_3 versus C_4 plants depends on temperature and CO_2 partial pressure (Ehleringer and Pearcy 1983; Ehleringer and Monson 1993). Ecosystems dominated by C_4 plants are those which display high temperatures during the growing season and aridity. They are also those which display low CO_2 partial pressure values as C_4 plants show anatomical and biochemical adaptations that allow them to concentrate the CO_2 in the leaf cells before carrying out photosynthesis (Ehleringer et al. 1997). On account of the high temperatures, C_4-dominated ecosystems appeared first in low latitudes before extending to higher latitudes. The change from C_3 dominated ecosystems to those dominated by C_4 plants was initially studied in paleosoil carbonates from the sediments of the Siwaliks (Pakistan) (Quade et al. 1992; Morgan et al. 1994). A sudden change in $\delta^{13}C$ was observed between 7 and 5 Ma. Subsequently, several studies have demonstrated that the expansion of C_4 plants can also be detected in other sections from Africa, Asia, North America, and South America (e.g., Quade et al. 1992; Morgan et al. 1994; Cerling et al. 1997; Latorre et al. 1997; Fox and Koch 2004; Wang et al. 2006). This expansion was initially proposed to have been a consequence of a global reduction in the atmospheric CO_2 concentration in the late Miocene (Cerling et al. 1993). Other authors have suggested that more gradual regional controls may have also played an important role on account of the lack of synchronicity in the global expansion of C_4 plants (Morgan et al. 1994; Pagani et al. 1999; Tipple and Pagani 2007).

Europe has been poorly represented in the global study of C_4 plant expansion as there are very few sections with a complete mammalian record spanning the Miocene–Pliocene boundary (Cerling et al. 1997). Nevertheless, the Teruel–Alfambra region in northeastern Spain (Fig. 1) constitutes an excellent location to study the vegetation shift that took place during the late Neogene as it contains thousands of remains of vertebrates from different paleontological sites with well-constrained ages. In this paper, we show enamel $\delta^{13}C$ and $\delta^{18}O$ values from the equid *Hipparion* from 19 paleontological sites spanning an age range between approximately 10.9 and 2.7 Ma.

Geological setting, samples, and methods

The Teruel–Alfambra region is located in the northern part of the Teruel Basin. This region belongs to the middle mountainous Mediterranean climatic domain. In the present day, the vegetation is fully dominated by C_3 plants, although a minor C_4 component (e.g., *Salsola vermiculata*) can be found in the Teruel–Alfambra region and in the nearby Ebro Basin.

The Teruel Basin is situated in the northeastern side of the Iberian Peninsula (Fig. 1) and is oriented NNE–SSW. The basin is ~100-km long and ~15-km wide and is filled by a complete succession spanning middle to late Neogene sediments which are more than 500 m in thickness (van Dam et al. 2001).

The Neogene succession from the Teruel–Alfambra region is bounded by siliciclastic and evaporitic formations

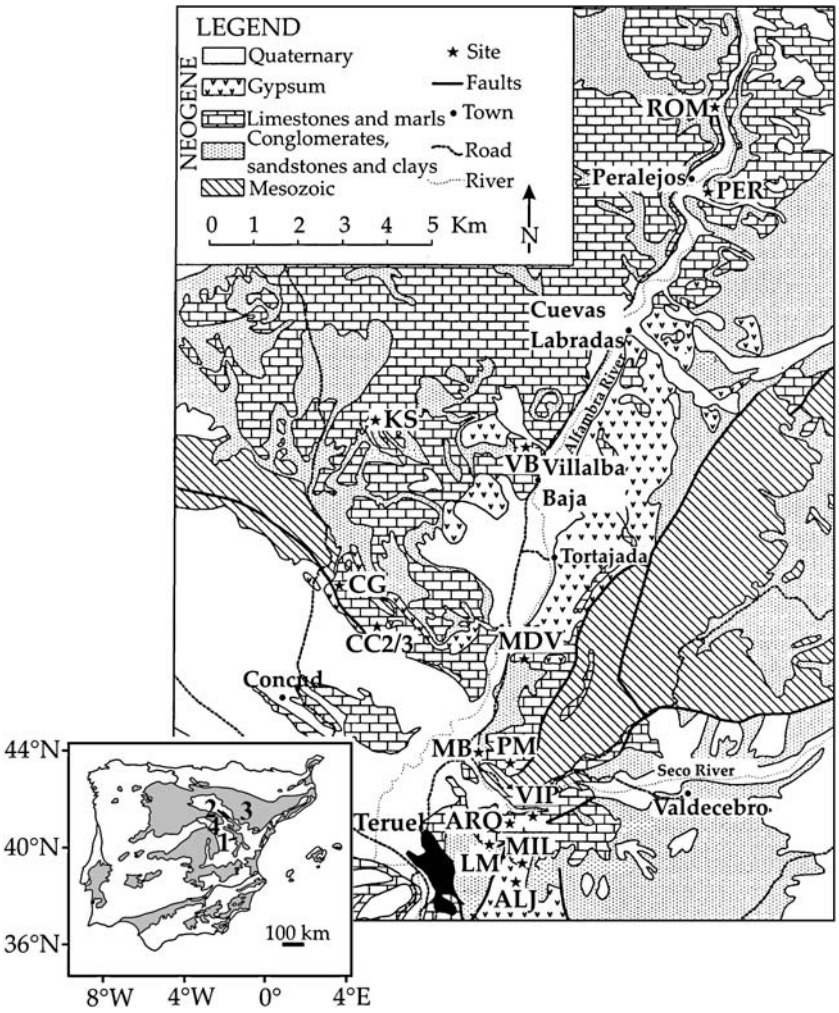

Fig. 1 Geological setting of the Teruel–Alfambra region (1 in the Iberian Peninsula map, which shows the location of Tertiary basins in *gray*) with the paleontological sites selected in this study: *ROM* La Roma 2, *MB* Masía del Barbo 2B, *VIP* Vivero de Pinos, *PM* Puente Minero, *ALJ* Los Aljezares, *MDV* Masada del Valle 2, *CC2* Las Pedrizas, *LM* Los Mansuetos, *CG* Cerro de la Garita, *ARQ* El Arquillo 1, *KS* Las Casiones, *MIL* Milagros, *PER* Peralejos E, *VB* Villalba Alta. (Modified from van Dam et al. 2001). The La Calera and Orrios sites do not appear on the map and are situated toward the South and North of the basin, respectively. The Nombrevilla 1 and Villarroya sites belong to the Calatayud and Ebro basins, respectively, meanwhile the Layna site is situated in the Iberian Range (2, 3, and 4, respectively, in the Iberian Peninsula map)

of Triassic age and also by Jurassic aged carbonate deposits. The oldest Neogene deposits are situated in the eastern part of the basin and are Early Miocene in age. In the middle part of the basin (Alfambra River valley), sediments are Late Miocene to Early Pliocene in age and represent alluvial fan distal facies and shallow lacustrine environments (van Dam et al. 2001). The Neogene deposits overlie unconformably upon Mesozoic and Paleogene formations. The dating method used in this area is mainly based on mammal faunas which are widely distributed across the basin (Alcalá 1994). The majority of the mammal localities are found in stratigraphic superposition. Several sections have also been dated by using magnetostratigraphic analysis (Alcalá et al. 2000). This allows correlation between the biostratigraphy of the mammalian succession and the geomagnetic polarity time scale (GPTS) of Cande and Kent (1995).

Tooth enamel samples, 134, from seven different species of the equid *Hipparion* (*Hipparion primigenium*, *Hipparion matthewi*, *Hipparion periafricanum*, *Hipparion laromae*, *Hipparion concudense*, *Hipparion fissurae*, and *Hipparion rocinantis*) were analyzed for $\delta^{13}C$ and $\delta^{18}O$. Samples came from 19 paleontological sites, 16 of which are located in the Teruel–Alfambra region, with the rest belonging to sites in the Calatayud and Ebro basins (Nombrevilla 1 and Villarroya) and the Iberian Range (Layna; Table 1 and Electronic supplementary material S1). Paleogeographic studies demonstrate that the architecture of the Northern Iberian Peninsula from 12 Ma ago to the present day has not changed substantially (Popov et al. 2004). Ages were assigned to the fossil sites (Table 1) according to Domingo et al. (2007), who carried out a biochronological study of the Upper Neogene of the Iberian Peninsula based on the fauna of macrommamals using the maximum likelihood appearance event ordination (ML AEO) method (Alroy 2000).

By using a single genus (*Hipparion*), vital effects (stable isotope fractionation due to different metabolisms), which could affect the interpretation of the isotope results, were reduced. Moreover, the analysis of enamel rather than bone should reduce diagenetic issues as enamel is less prone to diagenetic alteration compared to bone (Ayliffe et al. 1994; Lee-Thorp and Sponheimer 2003). Finally, when

Table 1 Biochronological ordination of the paleontological sites from the Teruel-Alfambra region used in this study and arranged by Domingo et al. (2007) using the *Maximum Likelihood Appearance Event Ordination* (ML AEO) method (Alroy, 2000).

Age (Ma)	Number of samples	Fossil sites	MN unit
2.720	4	Villarroya	MN16
3.912	7	Layna	MN15
4.180–3.580*	7	Villalba Alta	MN15
–	1	Peralejos E	MN14
4.186	8	Orrios 1	MN14
4.186	7	La Calera	MN14
5.689	7	Milagros	MN13
6.080	10	Las Casiones	MN13
6.319	19	El Arquillo 1	MN13
7.011	9	Cerro de la Garita	MN12
7.011	8	Los Mansuetos	MN12
7.011	3	Las Pedrizas	MN12
7.255	4	Masada del Valle 2	MN12
7.408	7	Los Aljezares	MN12
7.834	7	Puente Minero	MN11
8.040	6	Vivero de Pinos	MN11
8.789	7	Masía del Barbo 2B	MN10
8.789	5	La Roma 2	MN10
10.873	8	Nombrevilla 1	MN9

An absolute age could not be assigned to Villalba Alta (marked with an asterisk) by using this technique. Therefore, the paleomagnetic age proposed by Opdyke et al. (1997) and Oms et al. (1999) has been used. In the same way, an absolute age could not be attributed to Peralejos E where no paleomagnetic studies have been performed so far. Therefore, it has been placed in the chronological order as proposed by Domingo et al. (2007). Nombrevilla 1 and Villarroya belong to Calatayud and Ebro basins, respectively, and Layna corresponds to a karstic filling situated in the Iberian Range.

possible, third molars were used in this study to overcome paleoecological bias from juvenile development (Bryant et al. 1996).

The enamel was recovered by using a rotary drill with a diamond-tipped dental burr, and in some cases, by hand picking enamel under a binocular microscope from lightly crushed fragments. Enamel was removed from as large an area of the tooth as possible to avoid biasing the isotopic results with respect to seasonality, thus producing a homogeneous signal from several months or years. The material analyzed in this study is archived at Fundación Conjunto Paleontológico de Teruel (Dinópolis, Teruel, Spain), Museo Nacional de Ciencias Naturales (CSIC, Madrid, Spain), and Museo Geominero (Instituto Geológico y Minero de España, Madrid, Spain).

The carbon and oxygen isotopic results are reported in the δ-notation against VPDB. $\delta_{\text{sample}} = \left[\left(R_{\text{sample}} - R_{\text{standard}}\right)/R_{\text{standard}}\right] \times 1,000$; with $R = {}^{13}C/{}^{12}C$ and ${}^{18}O/{}^{16}O$. The analyses were conducted at the University of Plymouth (United Kingdom) using an acid digestion technique with a continuous helium flow GV IsoPrime with a multiflow preparation system. Approximately 4–5 mg of raw sample was weighed into individual, septum-sealed vials and placed in a hot plate maintained at 90°C. Each vial was automatically flushed with helium before excess H_3PO_4 was added. After approximately 24 h of equilibration time, the CO_2 was analyzed by continuous flow mass spectrometry. The in run NBS-19 standard gave a $\delta^{13}C$ value of 2.04±0.24‰ (VPDB; n=5) and a $\delta^{18}O$ value of −2.86±0.07‰ (VPDB; n=5). The NBS 120c Florida phosphate rock provided a $\delta^{13}C$ value of −6.21± 0.54‰ (VPDB; n=5) and a $\delta^{18}O$ value of −2.24±0.52‰ (VPDB; n=5). The reason why the samples were not pretreated prior to isotopic analysis was mainly due to the small amount of sample that was available. This omission of this stage should not have greatly affected our results as Koch et al. (1997) showed that the isotopic value of untreated enamel provides an average value among the isotopic values furnished by different types of pretreatment. Moreover, Rare Earth Element analyses carried out on the same samples indicated that no major diagenetical alteration has affected the *Hipparion* tooth enamel (Electronic supplementary material S2).

Results

Figure 2 (and Electronic supplementary material S1) shows $\delta^{13}C$ and $\delta^{18}O$ values measured on the tooth enamel of the equid *Hipparion* from the Teruel–Alfambra region, Calatayud and Ebro basins, and the Iberian Range. $\delta^{13}C$ values range between −13.3‰ and −8.5‰ (with a mean value of −11.6±0.8‰). $\delta^{18}O$ values range between −9.9‰ and −3.2‰ (with a mean value of −6.4±1.1‰). A similar trend exists between $\delta^{18}O$ and $\delta^{13}C$ values (i.e., toward an increase in the isotopic values). However, the correlation coefficient between these two variables is very low (r= 0.26) suggesting diagenesis has not brought about the homogenization of the isotopic values.

As far as $\delta^{13}C$ values are concerned, significant statistical differences by means of ANOVA tests have not been detected between different *Hipparion* species ($F_{6,\ 126}$= 1.732, p=0.119). This suggests that $\delta^{13}C$ values have not been biased on account of vital effects. Significant differences have also not been found when comparing different types of teeth (ANOVA, $F_{2,\ 123}$=0.534, p=0.588); therefore, the isotopic results are not affected by differences in the time of mineralization associated with each tooth.

In general, $\delta^{13}C$ values are typical for a C_3-based diet. The range of $\delta^{13}C$ values calculated for the plants, by using the dental enamel $\delta^{13}C$ values from the *Hipparion* and an isotopic fractionation of around −14‰ (Cerling and Harris 1999) is −26.2 to −23.4‰ (VPDB), which are the typical $\delta^{13}C$ values for C_3 plants (−34 to −23‰ VPDB). However,

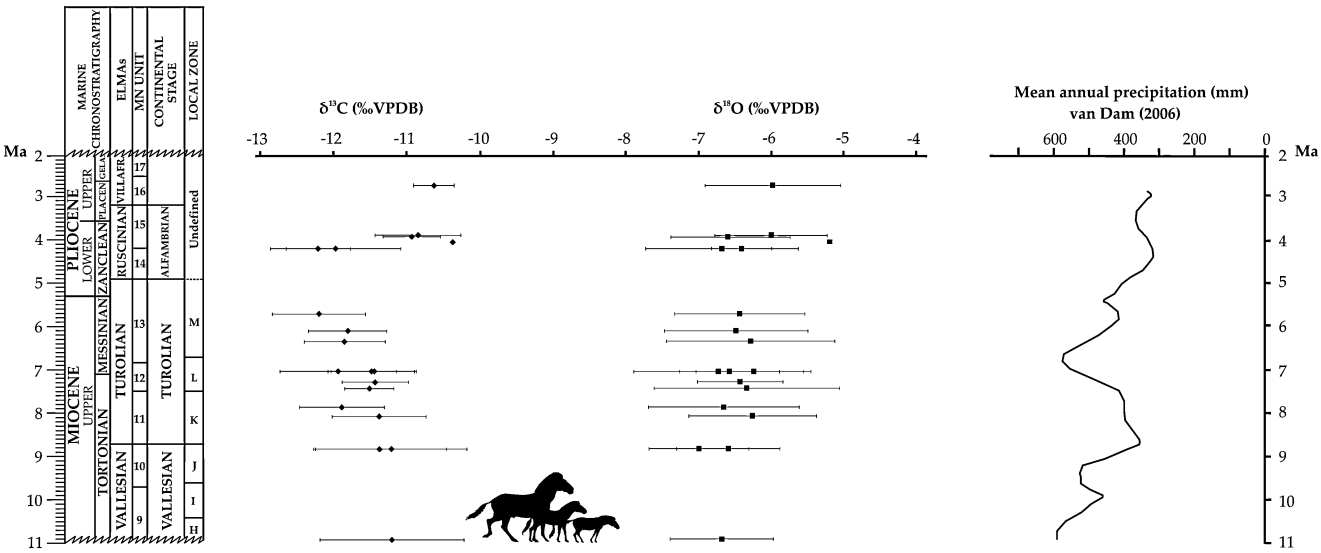

Fig. 2 Chronological scheme for the Miocene–Pliocene boundary in Spain and $\delta^{13}C$ and $\delta^{18}O$ values obtained in the *Hipparion* dental enamel from the Teruel–Alfambra region, Calatayud and Ebro basins and Iberian Range. The mean annual precipitation curve obtained by van Dam (2006) by studying micromammal assemblages from Europe is also shown. *Error bars* are ±1 SD. *Hipparion* species reconstruction by Israel M. Sánchez

statistical analyses show significant differences in the $\delta^{13}C$ between different sites (ANOVA, $F_{17,\ 115}=2.486$, $p=0.002$) and between Mammal Neogene (MN) units (ANOVA, $F_{7,\ 125}=5.285$, $p<0.001$) indicating that variations do exist in the carbon isotope signal recorded in the *Hipparion* enamel over time. The $\delta^{13}C$ values show a near-constant trend until the end of MN14 where a change has been pinpointed. In this case, a significant difference in $\delta^{13}C$ values before and after the MN14/MN15 boundary (Student *t* test, $t_{134}=3.616$, $p=0.002$) is evident. This positive isotope shift may be indicating, on the one hand, a slight increase in the consumption of C_4 plants by *Hipparion*, or on the other hand, a change in C_3 vegetation toward more open habitats, where the evaporation rate would have been higher after MN14. If C_4 consumption is responsible, then the percentage of C_4 plants consumed by each individual can be calculated using the equation proposed by Koch et al. (2004). Using this approach, the maximum C_4 consumption has been estimated at 27.5%, whereas the minimum is 0%.

Significant differences by means of ANOVA tests are not observed either in $\delta^{18}O$ values among species and types of teeth used ($F_{6,\ 125}=0.950$, $p=0.462$; $F_{2,\ 122}=0.029$, $p=0.972$, respectively) indicating that vital effects and time of tooth mineralization have not affected the isotopic signal. Contrary to what happens with $\delta^{13}C$ values, no statistically significant differences have been detected when considering $\delta^{18}O$ values between sites and MNs on the whole (ANOVA, $F_{17,\ 114}=0.381$, $p=0.987$; $F_{7,\ 124}=0{,}456$, $p=0.865$, respectively).

Changes in the $\delta^{18}O$ values of ingested water ($\Delta\delta^{18}O_{water}$) and mean annual temperature (ΔMAT) have been estimated by using the equations proposed by Iacumin et al. (1996) and Yurtsever and Gat (1981) which relate $\delta^{18}O_{CO3}$ and $\delta^{18}O_{water}$ and $\delta^{18}O_{water}$ and MAT (°C), respectively. An increase of ~0.7‰ has been calculated for $\delta^{18}O_{water}$ which corresponds to an increase of ~2.1°C in the MAT when comparing values from MN9 to MN16.

Discussion

The expansion of C_4 plants was initially attributed to a drop in the atmospheric CO_2 concentration in the Late Miocene (Cerling et al. 1993). However, Pagani et al. (1999) argued that the CO_2 concentration showed an increase from a minimum value at 14 Ma (~180 ppmv) up to values between 320 and 250 ppmv at the end of the Miocene (9 Ma). Therefore, if the mechanism that triggered the expansion of C_4 grasses was the decrease in atmospheric CO_2 concentrations, then the expansion should have occurred at the beginning of the Miocene. Regional factors or changes in the climatic conditions that could have also favored the expansion of C_4 plants, such as an increase in aridity and a change in the seasonal precipitation rate (precipitation during the warm season), are suggested as reasons for the lack of synchronicity observed in the expansion of these plants (Pagani et al. 1999; Fox and Koch 2004).

The tectonic evolution of the Himalaya has been traditionally invoked as playing a major role in the expansion of C_4 plants as it triggered profound changes in oceanic and atmospheric circulation resulting in an increase

in the seasonality of the precipitation and in aridity levels in mid and high latitude regions in the North Hemisphere (Pagani et al. 1999; Wang et al. 2006). This event brought about, likewise, an enhancement in the silicate-rock weathering resulting in the drawdown of atmospheric CO_2 (Ehleringer and Monson 1993).

Another global factor that may have driven a major climatic change is the closure of the Panama Isthmus. This gradual event took place between 13 and 2.6 Ma and resulted in a decrease in the mixing of Atlantic and Pacific waters that gave rise to the development of the modern Atlantic thermohaline circulation, an increase in the temperature, an enhanced evaporation rate in the North Atlantic, and an increase in the precipitation rates in Northern Hemisphere high latitudes (Haug and Tiedemann 1998; Haug et al. 2001; Lunt et al. 2008). The intensification of the Gulf Stream as a consequence of the reorganization of the ocean circulation introduced warm and saline waters to northern latitudes at ~4.6 Ma (Haug and Tiedemann 1998; Lear et al. 2003). This has been related to a warm period between 4.7 and 3.1 Ma, known as the Pliocene Warm Period (Haug et al. 2001).

Even though the tectonic evolution of the Himalaya and the closure of the Panama Isthmus constitute key factors in global oceanic and atmospheric conditions, it is necessary to look for additional regional factors related with the environmental shifts reflected by the isotopic analyses of the *Hipparion* dental enamel from the Teruel–Alfambra region. During the time period represented in this study (~10.9 to 2.7 Ma), tectonic and environmental changes of paramount importance were taking place in the Mediterranean area which gave rise to the Messinian Salinity Crisis (Hsü et al. 1973). It is generally accepted that the main cause of this crisis was the tectonic closure of the Mediterranean Basin that brought about a progressive reduction of the marine water circulation coming from the Atlantic and which culminated in the formation of thick evaporite deposits. Around 5.96 and 5.59 Ma, the deposition of the "Lower Evaporites" and "Upper Evaporites" and "Lago Mare" (Lake Sea) sediments took place, respectively (Krijgsman et al. 1999). The continuous increase in the ice-volume in the Antarctic since the Middle Miocene may have also played an important role in this crisis as it resulted in a drop in sea level which might have restricted oceanic circulation toward the Mediterranean Basin (Rouchy and Caruso 2006). This crisis had important climatic consequences that not only affected the marine realm, but also the terrestrial realm (Agustí et al. 2006; Fortelius et al. 2006; van der Made et al. 2006). Van Dam (2006) carried out a reconstruction of the precipitation rates in Europe between 12 and 3 Ma based on micromammal assemblages. He argued that after the relatively dry conditions of the Middle Miocene (16–13 Ma), an increase in the precipitation rate took place between 13 and 11 Ma (MN7-8), reaching a maximum at about 11–10 Ma (beginning of the Vallesian, MN9). Van Dam (2006) also proposed that a decrease in the precipitation rate between 10 and 5 Ma, whose minimum was at 4 Ma (Ruscinian, MN15), took place and resulted in an increase in aridity (Fig. 2). Although this author proposed that the tectonic evolution of the Himalaya was ultimately responsible for the increase in aridity in the Late Miocene and Early Pliocene in Europe, the Messinian Salinity Crisis may have played an important role, boosting the aridity pattern detected in the Western Mediterranean Basin. Furthermore, Tiedemann et al. (1989, 1994) pointed out that at ~4.6 Ma, an increase in aridity took place in the southern Sahara, a fact that could have also influenced the environmental conditions of the Iberian Peninsula.

The *Hipparion* dental enamel from the Teruel–Alfambra region has yielded $\delta^{13}C$ values typical of a diet based on C_3 plants. The significant increase detected at the MN14/MN15 boundary may be indicative of a slight increase in the consumption of C_4 plants. Nevertheless, Hernández Fernández et al. (2007) have shown the predominance of forested biomes in the late Neogene of the Iberian Peninsula, which hindered the development of C_4-dominated vegetation. Even though these kinds of plants have probably never been abundant in the Mediterranean region, Quade et al. (1994) argued that C_4 plants might have been represented in low proportions as can be deduced from $\delta^{13}C$ values obtained by these authors in Rhodes. Moreover, it should be noted that *Salsola vermiculata*, a C_4 plant of the Chenopodiaceae family, occurs nowadays in the Teruel–Alfambra region and in the nearby Ebro Basin. If the increase in $\delta^{13}C$ values shown by the *Hipparion* of the Teruel–Alfambra region is interpreted as suggesting an increase in the percentage of C_4 plants in the diet of this equid at ~4.2 Ma, then a slight expansion of C_4 plants in this area might have taken place subsequent to other regions of the world. This would support the notion that the expansion of these plants was controlled by regional climatic and environmental factors, ultimately driven by tectonically controlled changes in the global climate (Latorre et al. 1997; Cerling et al. 1997; Fox and Koch 2004). Cerling et al. (1997) proposed an age of between 8 and 6 Ma for the expansion of C_4 plants in Asia, Africa, South America, and North America based on the isotopic analyses of the dental enamel of different species of macromammals. Our study and those by Latorre et al. (1997), Passey et al. (2002), Fox and Koch (2004), and Ségalen et al. (2007) suggest that such expansion took place between 6 and 4 Ma at higher latitudes.

However, as pointed out before, the increase in $\delta^{13}C$ values may also be related to a change in the C_3 ecosystems toward more arid conditions (Quade et al. 1989). This

increase in the aridity pattern may be in response to the climatic and environmental changes produced after the Messinian Salinity Crisis which affected regional precipitation rates (Tiedemann et al. 1989, 1994; van Dam 2006) (Fig. 2). This explanation agrees well with a rise in the hypsodonty index detected in different species of *Hipparion* in the Teruel–Alfambra region (Electronic supplementary material S3; Pesquero 2003; Pesquero et al. 2006), as an adaptive process toward a more abrasive diet.

As far as $\delta^{18}O$ values are concerned, the gradual increase detected from MN9 to MN16 may be related to an increase in the MAT of ~2.1°C. Fluteau et al. (2003) carried out a reconstruction of the climatic conditions that took place as a consequence of the Messinian Salinity Crisis using the LMDz Atmospheric General Circulation model and detected an increase in the MAT of ~2.5°C in response to the desiccation of the Mediterranean Basin. Montuire et al. (2006) also estimated MAT values based upon fossil rodent assemblages in Europe from MN9 to MN15. Some of the sites considered by these authors are situated in the Teruel–Alfambra region or in its immediate vicinity (Nombrevilla, Alfambra, Los Aljezares, and La Gloria 4). The increase in MAT values obtained by Montuire et al. (2006), ~3.4°C, is not far from the one obtained from the *Hipparion* enamel in this study. Preliminary results based on the bioclimatic analysis of rodent faunas from the Iberian Peninsula have also shown a significant increase in temperature from MN13 to MN14 (Gómez Cano et al. unpublished data). This increase in $\delta^{18}O$ values during the Mio–Pliocene transition has also been detected on a global scale in Asia (Quade et al. 1992), Europe (Quade et al. 1994), North America (Fox and Koch 2004), South America (Latorre et al. 1997), and Africa (Morgan et al. 1994), with the most significant change being observed between 8 and 4 Ma. Most studies usually agree that the increase in $\delta^{18}O$ values registered in the dental enamel and paleosoil carbonates at this time might be indicative of an increase in the MATs and/or an increase in aridity pattern (as shown by $\delta^{13}C$ values; Latorre et al. 1997; Tipple and Pagani 2007 and references therein).

In summary, we have performed stable isotope analyses ($\delta^{13}C$ and $\delta^{18}O$) on the *Hipparion* dental enamel from the Teruel–Alfambra region spanning a broad time interval (from 10.9 to 2.7 Ma). Up to now, Europe had been poorly sampled with the aim of gaining information about the environmental conditions that took place across the Miocene–Pliocene boundary, and therefore, this new study provides valuable information for future comparative works. Our data show an increase in $\delta^{13}C$ values at ~4.2 Ma that may be related either to a slight increase in the C_4 vegetation content and/or to a shift in the C_3 ecosystems toward more open habitats at this period of time. Although several contemporary global and regional events that could have triggered the environmental change suggested by our isotopic results have been explored, the ultimate causes that gave rise to it are currently difficult to assess.

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The experiments carried out in this research comply with the current laws of Spain and the United Kingdom.

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